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Supplementary notes:

Discussion about the bias for the forward direction over the backward one in the selectivity histograms.

The selectivity histograms in Fig. 1i and Extended Data Fig.3d show that the numbers of apical dendritic branches and somata of layer 5 (L5) pyramidal neurons active during forward running are larger than those during backward running. We found that on apical tufted dendrites, the number of dendritic spines active during forward running are also larger than during backward running (Extended data Fig. 5f; 42% forward-specific, 32% backward-specific, and 26% overlapping; *n* = 98 spines from 12 dendrites imaged over 2.5 minutes in mice running in each direction). This suggests that the bias in the selectivity histograms is likely related to a higher level of synaptic input activities to L5 pyramidal cells during forward running than during backward running. The reasons for this difference in synaptic activities between the two running modes remain to be determined. Forward and backward running engage different patterns of muscle activity. The increase in the stride length is greater during forward than during backward running after mice were trained for 40 minutes (Fig. 1c and Extended Data Fig.1d). These differences indicate that the patterns of neuronal activities in the spinal cord differ between forward and backward running. Previous studies have shown that neurons in cat primary motor cortex receive different inputs from spinal cord during forward and backward walking on a treadmill[1]. It is likely that different inputs coming directly from these spinal cord neurons contribute, at least in part, to the differences in the level of neuronal activities detected in apical tufted dendrites and L5 somata.

It is worth mentioning that the region of the primary motor cortex we imaged is ~200 μm in square and is centered at 0.5 mm anterior from bregma and 1.2 mm lateral from the midline. This corresponds to the forelimb motor cortex[2,3]. Because the forelimbs are involved in both forward and backward running, the bias in dendritic and L5 soma activity to forward running is likely due to differences in input activities rather than the specific region we imaged.

Discussion about the example traces in Fig. 2b.

Unlike the spine 1, the peaks of Ca^{2+} fluorescence in spines 2, 3, and 5 were lower than that of dendritic shaft Ca^{2+} fluorescence at the time of spike generation. The elevations of Ca^{2+} in these spines were due to the influx from the dendritic Ca^{2+} spike and therefore did not reflect calcium influx induced by synaptic inputs (Extended Data Fig. 5k-l; please see also the reference Chen et al. (2013)[4]). Because these spines were not active at the time of Ca^{2+} spike generation, they did not get potentiated. The peak amplitude of the spine 4 is slightly higher than that of the dendritic spike at the dendritic shaft. However, the average peak amplitude of this spine is not higher after the Ca^{2+} spike generation than before. This suggests that spine potentiation is affected not only by Ca^{2+} spikes but also influenced by other factors such as the peak amplitude of spine Ca^{2+} activity (Fig. 2e). Nevertheless, our data in Fig. 2 show that synchronization with Ca^{2+} spikes plays a key role in the potentiation of spines when they are active at the time of Ca^{2+} spike generation.

Discussion about spine depotentiation when spines are active within 5 seconds before the spikes.

Several lines of evidence suggest that the depotentiation of spines active within 5 seconds before the spikes is due to the interactions between active spines and the local Ca^{2+} spike. First, we found that in the task switching experiment, the depotentiation occurs only in spines active within 5s of spike generation during backward running (Fig. 3a-c). The peak amplitude of spine Ca^{2+} transients did not show significant changes if spines were active more than 5s before spike generation or did not encounter a spike during backward running (Fig. 3c and Extended Data Fig. 7c-e). Second, we found that the peak amplitudes of spine Ca^{2+} transients were comparable within the first and second half of a 30s forward running trial, either before or after task-switching. This rules out the possibility that spine depotentiation is due to a gradual decrease of spine Ca^{2+}transients during the 30s forward running trial before task switching and a sudden increase after the same task is switched back (Extended Data Fig. 7f). Third, in Fig. 4, we found that previously potentiated spines underwent depotentiation during backward running only when they were active within 5s before backward running-induced spikes. In this experiment, both spine transients and the Ca^{2+} spike were measured during the period of backward running. The observed depotentiation was not related to task-switching. Taken together, these results suggest that the depotentiation of spine Ca^{2+} transient peak amplitudes in Figs.3 and 4 is due to the interactions between asynchronously activated spines and Ca^{2+} spikes.

Discussion about the potential role of backpropagating action potentials on calcium spike generation

Our data shows that local injection of tetrodotoxin (TTX) in layer 5 (L5) blocked the activity of L5 soma, but did not block the generation of dendritic Ca^{2+} spikes in tuft dendrites in L1 and the activity in apical dendritic trunks (Fig. 5b,c). On the other hand, TTX injection in L1 prevented the generation of Ca^{2+} spikes in apical tufted dendrites (Fig. 5b). These results indicate that backpropagating action potentials (bAPs) from L5 soma are not required for the generation of Ca^{2+} spikes at apical tuft branches. To confirm that TTX injection in L5 or L1 is local, Texas red dye (mimicking TTX injection) was injected in L5 or L1. Texas red spread to a region 237 ± 19 µm (L5) or 188 ± 10 µm (L1) in diameter. Importantly, the dye injected in L5 did not spread to L1 and vice versa (Extended Data Fig. 9a-b).

Our observations that bAPs from L5 somata are not required for Ca^{2+} spike generation on apical tuft branches is consistent with previous studies showing that direct stimulation of inputs on the tufted branches induces the generation of dendritic spikes, which propagate down along the apical trunk to cause the depolarization at somata[5,6]. Furthermore, several lines of evidence suggest that bAPs from L5 soma rarely invade distal apical dendrites[7,8]. Taken together, these findings suggest that branch-specific generation of dendritic spikes is mainly caused by the activities of synaptic inputs (both excitatory and inhibitory) onto individual tuft branches of L5 pyramidal neurons.

While our data suggest the important role of local synaptic inputs in dendritic Ca^{2+} spike generation at the tufts, bAPs from L5 somata could enhance the occurrence of Ca^{2+} spikes. Recent studies been shown that both apical trunk depolarization and synaptic activation at tuft branches are important for triggering Ca^{2+} spikes at apical tuft branches of L5 pyramidal cells in

the mouse somatosensory cortex[5,9]. bAPs could cause the depolarization at the trunk nexus which could in turn enhance the generation of Ca^{2+} spikes on apical tuft branches.

Discussion about the impact of the deletion of somatostatin (SST)-expressing interneurons on animals' behaviors.

All of our imaging and behavioral experiments with SST deletion were performed within the first 2 days of SST deletion (3 doses of Diphtheria toxin (DT) given 12 hrs apart starting on day 1; imaging and behavioral testing on day 3). We found that 69 ± 4% of SST neurons were deleted during this time period (*n* = 5 mice). Somewhat unexpectedly, SST-deleted mice did not show obvious disruptions on motor control over these 2 days. SST-deleted mice exhibited walking, running, and eating similar to the control (injected with DT) mice in the same cage. Importantly, the performance improvement in SST-deleted mice during forward treadmill running, as measured by the increase in the stride length, was comparable to that of control mice (Fig. 5g). To further evaluate the effect of SST deletion on the motor control, we examined the behavioral performance of SST-deleted mice on an accelerated rotarod. In this motor skill learning paradigm, mice learn to change their gait patterns in order to avoid falling off from the rotating rod[10]. We found that the performance improvement after 40 forward running trials (~40 minutes) was also comparable between SST-deleted mice and the non-deleted control mice (Extended Data Fig. 10a). This finding provides additional evidence that within 2 days after SST neurons deletion, mice do not lose their motor control in motor tasks involving running and balancing etc.

It is worth to mention that ~7 days after DT treatment to activate DTR, the majority of SST-deleted mice (>90%) develop seizures and eventually died. This suggests that over extended periods of time, SST interneurons are important for controlling the overall excitability of neuronal networks that is vital for the animal's survival. It is also worth to note that previous studies have shown that performance improvement on the rotarod is reduced 2-3 days after deleting microglial cells in mice[11]. As microglia comprise ~5-10% of cells in the CNS, this result, together with the data from SST-deleted mice, suggest that the effects of cell deletion on animal's motor behavior depend on cell types and the time when behavioral tests are performed after the deletion.

Supplementary note references:

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